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Indeterminate Growth of Pericarps in Three Members of the Rhodymeniales (Rhodophyta)

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NOTE

INDETERMINATE GROWTH OF PERICARPS IN THREE MEMBERS OF THE RHODYMENIALES (RHODOPHYTA)[1,2]

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ABSTRACT

Pericarps generally are thought of as structures having determinate growth, serving as a protective covering for the developing gonimoblast. In laboratory culture, the pericarps of Champia parvula *(C. Agardh) Harvey,* Lomentaria baileyana *(Harvey) Farlow and* Lomentaria *sp. exhibited indeterminate growth. These tissues could be excised and grown as separate female plants. The new plants were indistinguishable from the parent tissue, were fertile, and produced viable carpospores.*

Key index words: Rhodymeniales; Champia parvula; Lomentaria baileyana; Lomentaria clavellosa; *pericarp; cystocarp; indeterminate growth; vegetative growth; regeneration.*

The pericarpic tissue of members of the Rhodophyta consists of gametophytic tissue originating from the female (Fritsch 1959). It is generally accepted that this tissue is determinate in growth. However, in three members of the Rhodymeniales, *Champia parvula, Lomentaria baileyana* and *Lomentaria* sp. (probably *L. clavellosa,* work is in progress to clarify the taxonomy) pericarpic tissue continued to grow after spore production. This is the first published report of indeterminate growth of pericarp tissue.

One Korean and two North American clones of *Champia parvula* (C. Agardh) Harvey, as well as one clone of both *Lomentaria baileyana* (Harvey) Farlow and *Lomentaria* sp. were studied. In addition, 10 morphological and 5 color mutants of one of the *C. parvula* clones were also examined. Unialgal cultures were maintained in 450 mL aerated glass bottles containing 400 mL of a one-fourth strength modified medium 'f' (Guillard and Ryther 1962). The medium consisted of 37.5 mg $NaNO_3$, 2.5 mg $NaH_2PO_4 \cdot H_2O$, 2.18 mg EDTA (disodium salt of ethylenediamine tetraacetic acid) and 0.33 mg Fe per litre of cartridge filtered (15 μm charcoal filtered and 0.3 μm Balston filter), autoclaved seawater. The iron stock solution was prepared by dissolving 1 g iron power in 10 mL of concentrated HCl and diluting to 1 L with deionized water. Media were changed once a week. Illumination was from the side with ca. 50 $\mu E \cdot m^{-2} \cdot s^{-1}$ of Cool White fluorescent light. *C. parvula* was grown at a temperature of 24° C on a 16:8 h L:D cycle. *Lomentaria baileyana* was maintained at 20° C and *Lomentaria* sp. at 16° C, both were on a 12:12 h, L:D cycle. The salinity was 30‰.

Under the above culture conditions, male gametophytes pro-

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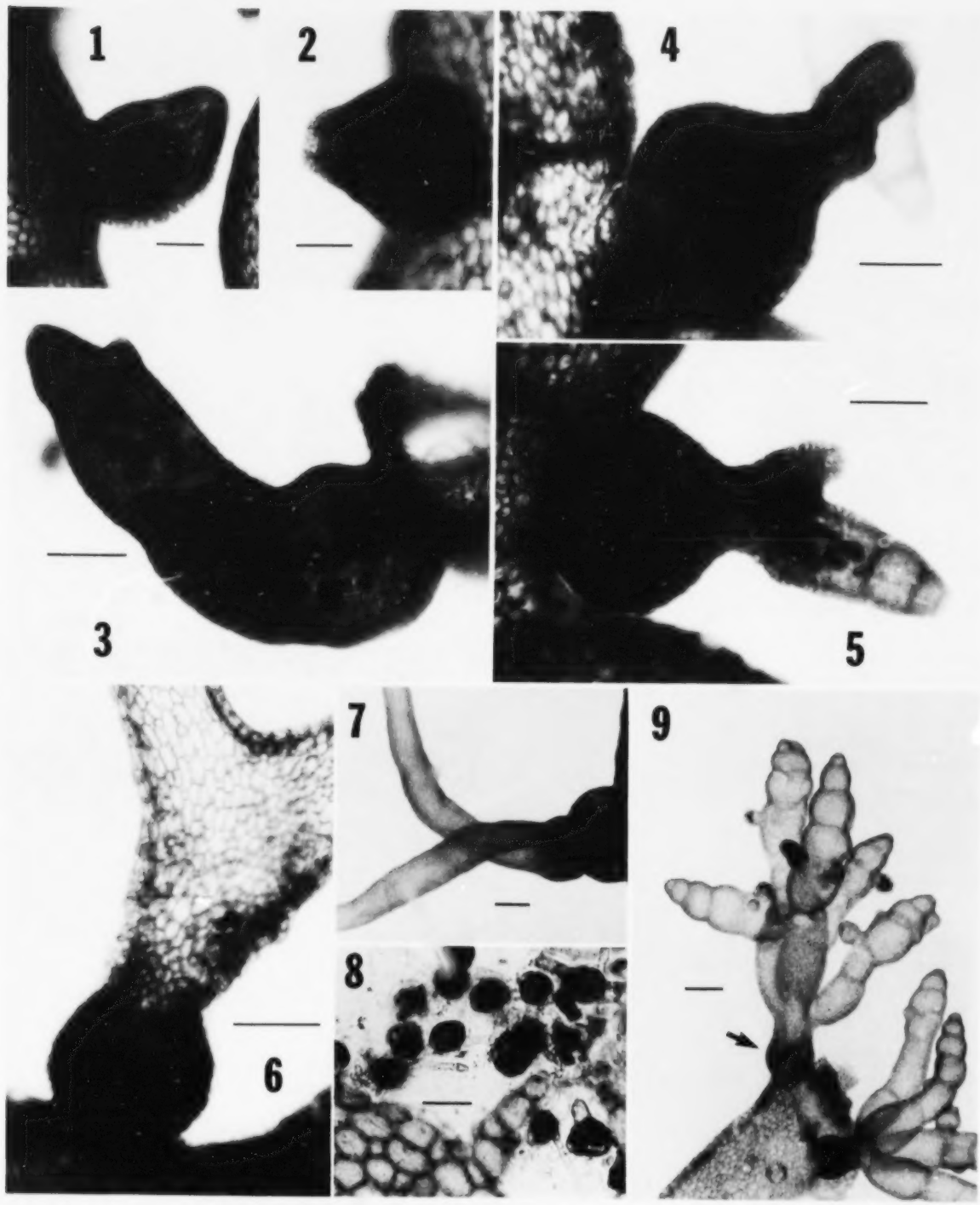
FIG. 1–9. Photomicrographs of indeterminate pericarp growth. FIG. 1. Pericarp of *Champia parvula* mutant with no ostiole. Scale bar = 0.25 mm. FIG. 2. Normal pericarp of *Champia parvula* showing prominent ostiole. Scale bar = 0.25 mm. FIG. 3. Growth of pericarp



of *Champia parvula* mutant. Carpospores are released internally. Scale bar = 0.5 mm. FIG. 4. Single branch at apex of normal *Champia parvula* pericarp. Ostiole is still functional. Scale bar = 0.5 mm. FIG. 5. Two branches at apex of normal *Champia parvula* pericarp. Ostiole is occluded and carpospores are released internally. Scale bar = 0.5 mm. FIG. 6. Later stage of growth of pericarp from normal *Champia parvula*. The remnant of the pericarp is still evident as a bulbous base. Scale bar = 0.5 mm. FIG. 7. Branches at apex of pericarp of *Lomentaria* sp. Scale bar = 0.5 mm. FIG. 8. Germinated carpospores from internally released spores of *Champia parvula*. Scale bar = 100 μm. FIG. 9. Re-fertilized tissue of indeterminate growth from pericarp of *Champia parvula*. The original pericarp is still evident as a bulbous base (arrow). Scale bar = 0.5 mm.

duced spermatia continuously and female gametophytes were always receptive. Cystocarps were obtained by co-culturing male and female plants starting with 0.5 to 1.0 cm branch tips. Cystocarps appeared within 8 to 10 d.

Experiments were performed with modified culture conditions to determine if the indeterminate growth could have been caused by culture conditions. Plants with mature cystocarps were used. Variations consisted of shaking (100 rpm rotary shaker + 150 mg $NaHCO_3 \cdot L^{-1}$) instead of aerating, temperatures of 16, 20 and 24° C and light : dark cycles of 8:16 h, 12:12 h and 16:8 h. Only one Rhode Island wild type *C. parvula* clone and *Lomentaria* sp. were grown under these conditions.

The first isolate demonstrating indeterminate growth of pericarp tissue was one of the morphological mutants (petite and stubby) of *Champia parvula*. The cystocarps of this isolate were unique in that they had no ostioles (Fig. 1). All other isolates of *C. parvula* had emergent cystocarps with prominent ostioles (Fig. 2). Approximately two weeks after a cystocarp was mature (carpospores present), the pericarp began to grow as if it were a normal branch (Fig. 3). At first it appeared that the gonimoblast might be growing also, but this turned out to be release of carpospores into the internal, hollow space of the thallus. Eventually the new growth became indistinguishable from the parent plant and could be excised for vegetative propagation.

Since the indeterminate growth was observed in a mutant, it was thought that this phenomenon was a characteristic of the mutant, especially since its cystocarps were also aberrant (i.e. no ostiole). However, when wild type clones of *C. parvula* (two from Rhode Island and one from Korea) were examined for this phenomenon, the pericarpic tissue of their cystocarps also exhibited indeterminate growth. These isolates only formed branches around the ostiole, instead of the entire pericarp growing. If only one branch formed, then the ostiole was still functional (Fig. 4). However, if two or more branches formed, then the ostiole was usually occluded (Fig. 5). As with the petite, stubby isolate, the new tissue was eventually indistinguishable from the parent thallus, except for the presence of a bulbous base—the remnant of the pericarp (Fig. 6). Nine other morphological mutants and five color mutants of *C. parvula* also were examined. Each showed the same pattern as the wild type clones. Two species of a closely related genus, *Lomentaria*, were also examined. Both *Lomentaria* sp. (Fig. 7) and *L. baileyana* showed the same kind of growth at the crown of the pericarp as the wild type clones of *C. parvula*.

When an ostiole did not form, or was later blocked, the carpospores germinated in situ (Fig. 8). Since in situ germination was possible it also was possible that the observed indeterminate growth of the pericarp tissue was only a germinated spore. However, in all cases the indeterminate growth was itself fertile, verifying that it was female tissue (Fig. 9).

Modified culture conditions did not affect the phenomenon of indeterminate growth. Under all combinations of temperature and photoperiod tested, pericarps eventually exhibited indeterminate growth in both *C. parvula* and *Lomentaria* sp. Plants cultured on a shaker gave the same response as plants in aerated cultures.

The phenomenon of indeterminate growth of pericarpic tissue has also been found in the field for *Lomentaria* sp. However, the ecological significance of this is not clear. One could hypothesize that this would increase the available area for reproduction, however, two problems arise. First, plants of both genera branch profusely and form many cystocarps per branch. Second, the ostiole is often blocked, which would be a definite disadvantage for carpospore dispersal.

There is no obvious distinction between the individual cells of the pericarps in these plants and cells of normal branches. The pericarpic cells are totipotent. Whatever controls the cooperation of these cells in forming pericarpic tissue and keeps them pericarpic, is lost as they age, and the cells assume the behavior of any other cortical cell, including the ability to resume meristematic activity and form branches. If germinated pericarps are excised, then new plants are formed that are indistinguishable from the parent plants. Totipotency, however, is not restricted to pericarpic tissue. Older tissue of *Champia* and *Lomentaria* will also produce new branches, under conditions of nutrient deprivation.

Mentioning of product names does not imply endorsement by the U.S. Environmental Protection Agency.

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